Original Article

Oral candidal carriage and species identification among betel quid chewers and oral submucous fibrosis patients

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Abstract

**Background:** The Candida species are saprophytic component of the normal oral microbiota. The disequilibrium in the homeostasis between Candida, host immune system and normal oral bacterial flora promotes a Candida carriage.

**Aim of the Study:** The present study was done to assess oral candidal carriage and species identification among healthy subjects, betel quid chewing (BQC), and oral submucous fibrosis (OSF) patients using CHROMagar® media.

**Materials and Methods:** Oral swabs collected from the reservoirs of Candida species (buccal mucosa and tongue) in study groups using sterile cotton swabs were inoculated on Sabouraud’s dextrose agar candidal medium and CHROMagar® Candida agar medium consecutively. Determination of Candida species was done based on morphology characteristics and pigment produced. The results were statistically analyzed using Chi-square test.

**Results:** Candida carriage was more in samples of buccal mucosa followed by the tongue. Candida albicans was the major isolate in both healthy subjects and BQC but with greater frequency in BQC. Candida tropicalis was the major isolate in OSF patients predominantly in buccal mucosa followed by the tongue.

**Conclusion:** C. tropicalis, a non-C. albicans species, was the major isolate in OSF. This unique finding implicates an immunological change of oral environment brought about by BQC and OSF.

Keywords
Betel quid chewing, Candida albicans, Candida tropicalis, CHROMagar®, oral submucous fibrosis

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Introduction

The Candida genus belongs to a heterogeneous cluster of fungi of the class deuteromycetes.[1] The Candida species are saprophytic component of the normal microbiota in gastrointestinal tract, skin, and vagina.[2] In the oral cavity, the Candida niches include the tongue followed by palate and buccal mucosa.[3] Candida carriage shows variation according to the age and health of an individual. Candida albicans is the predominant isolate with a mean carriage of about 18% and 41% in normal individuals and patients, respectively.[4]

The disequilibrium in the homeostasis between Candida, host immune system and normal oral bacterial flora favors Candida virulence factors such as the ability to form biofilm, various mechanisms to resist host defenses and production of hydrolytic enzymes such as proteases, phospholipases, and hemolysin which cause damage to the tissues.[5] Candida causes mycotic infections in both immunocompetent individuals and immunocompromised hosts.[6,7] The increase in the incidence of infections in immunocompromised individuals is due to their greater adaptability to divergent host niches.[8]

In recent years, worldwide, the consumption of tobacco products by youngsters in various forms has increased. The betel quid chewing (BQC) habit is found to be more prevalent in several Asian and South-Asian countries.[9-11] Oral submucous fibrosis (OSF), a chronic potentially malignant disorder, is of multifactorial origin with arecanut chewing as a predominant causative agent.[12] The tobacco contents (nicotine, polycyclic aromatic hydrocarbons, polonium, and nitrosoproline), which constitute as an additive component of betel quid, are reported to provide nutrition for Candida and promote their proliferation.[13] They may also increase the colonization of Candida by causing an increase in epithelial keratinization, decrease in salivary immunoglobulin A and leukocytic function, and oral epithelial changes such as atrophy, hyperplasia, and dysplasia which may compromise the epithelial integrity.[14]
A shift in the colonization of non-\( C. \text{albicans} \) species and their emergence as major potential pathogens can be related to their virulence factors, the increase in the population at special risk and use of antibiotics.\(^{[13]} \) The recognition of \( C. \) isolates is of importance in the better understanding of host-pathogen interaction in the pathogenesis of disease and treatment planning. The categorization of \( C. \) isolates can be done by various chromogenic media. Chromogenic media are species specific and yield candidal colonies with varying pigmentation due to the formation of secondary substrates by reacting with \( C. \) species-specific enzymes. CHROMagar\(^{®}\) is a chromogenic media known to identify and differentiate species based on color and colonial characteristics.\(^{[16]} \)

This study is focused to assess oral candidal carriage and species identification among healthy subjects, BQC, and OSF patients using CHROMagar\(^{®}\) media.

**Materials and Methods**

The study was done after obtaining permission from the Institutional Review Board. The study participants informed consent was taken before the collection of the sample, and a detailed case history was recorded.

**Inclusion criteria**

The study participants were in the age group of 20-45 years with equal males and females in the groups (M: F=1:1). They were categorized into three groups.

- Group A: Healthy individuals without BQC habit \((n = 5)\)
- Group B: BQC with a mean duration of BQC habit of 15-25 years and frequency of 5-6 times/day. \((n = 15)\)
- Group C: OSF with a history of BQC for a mean duration of 15-25 years with a frequency of 5-6 times/day. \((n = 15)\).

**Exclusion criteria**

The study excluded individuals:

- With the concurrent habit of tobacco smoking, alcohol consumption, areca nut and gutka chewing
- Using antifungal agents, antibiotics, non-steroidal anti-inflammatory drugs/steroids within the past 12 weeks
- With systemic disorders such as diabetes mellitus, hepatitis B, hepatitis C, HIV and acquired immunodeficiency syndrome
- Denture wearers.

**Sample collection**

Oral swabs were collected from the most common oral reservoirs of \( C. \) species (buccal mucosa and tongue) using sterile cotton swabs in all study participants.

**Candida species identification**

The oral swabs were inoculated onto sabouraud’s dextrose agar (SDA) (Himedia\(^{®}\)) and incubated at 37°C for 48 h. Candidal colonies grown on SDA were inoculated into CHROMagar\(^{®}\) Candida agar medium. The inoculated plates were incubated at 37°C and observed up to 7 days. \( C. \) species recognition was done based on the morphology of the colonies and color produced by the chromogenic reaction between secondary substrates present in chromogenic media containing glucosaminidase substrate and enzymes of \( C. \) species. \( C. \text{albicans}, C. \text{tropicalis}, C. \text{krusei}, \) and \( C. \text{glabrata} \) were identified by the production of green, dark blue, pink, and pink with darker mauve center colored colonies, respectively [Figure 1].\(^{[16]} \)

**Results**

**Buccal mucosa**

In buccal mucosa of healthy subjects, 60% of the samples showed candidal growth and the only candidal isolate was \( C. \text{albicans}. \)

In BQC, the \( C. \) species isolated from the buccal mucosa was as follows: \( C. \text{albicans} \) (66.7%), \( C. \text{krusei} \) (20%), \( C. \text{albicans} \) and \( C. \text{krusei} \) (6.7%), and no colonies (6.7%). \( C. \text{albicans} \) was the major isolate in BQC followed by \( C. \text{krusei}. \)

The \( C. \) species isolated in buccal mucosa of OSF patients was as follows \( C. \text{tropicalis} \) (80%), \( C. \text{albicans} \) (13.3%), \( C. \text{krusei} \) (6.7%), \( C. \text{albicans} \), and \( C. \text{krusei} \) (6.7%). \( C. \text{tropicalis} \) was the major isolate in OSF group followed by \( C. \text{albicans} \) and \( C. \text{krusei} \) [Figure 2 and Table 1].

**Tongue**

In healthy subjects, 60% of the samples collected from tongue showed candidal growth and the predominant \( C. \) species isolated was \( C. \text{albicans}. \) This finding mirrored the buccal mucosa site.

In BQC, the distribution of \( C. \) species was as follows: \( C. \text{albicans} \) (26.7%), \( C. \text{krusei} \) (20%), \( C. \text{albicans} \) and \( C. \text{krusei} \) (6.7%), and no colonies (46.7%). \( C. \text{albicans} \) was the major isolate in BQC followed by \( C. \text{krusei}. \)

The \( C. \) species isolated in OSF patients was as follows: \( C. \text{tropicalis} \) (60%), \( C. \text{albicans} \) and \( C. \text{krusei} \) (20%), and \( C. \text{albicans} \) and \( C. \text{krusei} \) (13.3%). \( C. \text{tropicalis} \) was the major isolate in OSF patient followed by \( C. \text{albicans} \) and \( C. \text{krusei} \). No colonies were isolated in 6.7% of the samples [Figure 3 and Table 2].

Candidal isolate was more in samples of buccal mucosa compared to \( C. \) isolates in the tongue. \( C. \text{albicans} \) was the major isolate in healthy subjects and BQC and showed an increase in frequency in BQC compared to healthy subjects.

\( C. \text{tropicalis} \) was the major isolate in OSF patients in both oral \( C. \) reservoirs, predominantly in buccal mucosa followed by tongue. Statistically, the above observations were found to be highly significant. Among 35 total cases, Fisher’s exact test value was 29.125 with \( P < 0.001 \) in buccal mucosa and 22.884 with \( P < 0.001 \) in tongue.
The homeostasis between Candida, host immune system and normal oral bacterial flora determines the role of Candida species as either saprophytes or opportunistic pathogens in the oral cavity. The prevalence of Candida isolates in oral cavity is regulated by endogenous factors such as: (a) Oral epithelial cell antimicrobial peptides such as defensins, cathelicidins and histidine, and epithelial integrity, (b) salivary constituents such as salivary immunoglobulin A, lysozyme, histidine-rich polypeptides, lactoferrin, and lactoperoxidase, and (c) oral cavity temperature and exogenous factors such as high carbohydrate diet. The epithelial cells provide a greater surface area which may promote Candida adhesion. The high carbohydrate diet is also reported to facilitate Candida adherence to epithelial cells by reduction in pH due to degradation of carbohydrate in saliva. The earlier in-vitro studies have reported C. albicans to have greater adhesion to oral epithelial cells followed by C. tropicalis and C. parapsilosis. The presence of more α-L-fucose remnants promotes greater adhesion of C. albicans. C. albicans is major isolate in healthy subjects on the tongue and buccal mucosa in the present study is in consonance with previous observations.

BQ with tobacco chewing is reported to cause an increase in salivary pH of 8-10 due to chemical constituents such as slaked lime and nicotine, a component of tobacco. C. albicans is able to adapt to pH of 2-10 by actively alkalizing surrounding environment, release of ammonia after amino acid degradation and by phenotypic switching. C. albicans biofilms are also resistant to neutrophils due to the presence of β-glucans in the extracellular matrix.

### Table 1: Distribution of Candida species on buccal mucosa site in each group

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Healthy subjects, n=5</th>
<th>BQC, n=15</th>
<th>Oral submucous fibrosis, n=15</th>
<th>Fisher’s exact test value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>Count</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>29.125</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>60.0</td>
<td>66.7</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>C. albicans and C. krusei</td>
<td>Count</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>0.0</td>
<td>6.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>C. krusei</td>
<td>Count</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>0.0</td>
<td>20.0</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>Count</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>0.0</td>
<td>0.0</td>
<td>80.0</td>
<td></td>
</tr>
<tr>
<td>No colonies</td>
<td>Count</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>40.0</td>
<td>6.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>5</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>


**Discussion**

The homeostasis between Candida, host immune system and normal oral bacterial flora determines the role of Candida species as either saprophytes or opportunistic pathogens in the oral cavity. The prevalence of Candida isolates in oral cavity is regulated by endogenous factors such as: (a) Oral epithelial cell antimicrobial peptides such as defensins, cathelicidins and histidine, and epithelial integrity, (b) salivary constituents such as salivary immunoglobulin A, lysozyme, histidine-rich polypeptides, lactoferrin, and lactoperoxidase, and (c) oral cavity temperature and exogenous factors such as high carbohydrate diet. The epithelial cells provide a greater surface area which may promote Candida adhesion. The high carbohydrate diet is also reported to facilitate Candida adherence to epithelial cells by reduction in pH due to degradation of carbohydrate in saliva. The earlier in-vitro studies have reported C. albicans to have greater adhesion to oral epithelial cells followed by C. tropicalis and C. parapsilosis. The presence of more α-L-fucose remnants promotes greater adhesion of C. albicans. C. albicans is major isolate in healthy subjects on the tongue and buccal mucosa in the present study is in consonance with previous observations.

BQ with tobacco chewing is reported to cause an increase in salivary pH of 8-10 due to chemical constituents such as slaked lime and nicotine, a component of tobacco. C. albicans is able to adapt to pH of 2-10 by actively alkalizing surrounding environment, release of ammonia after amino acid degradation and by phenotypic switching. C. albicans biofilms are also resistant to neutrophils due to the presence of β-glucans in the extracellular matrix.
Candida species in betel quid habit associated conditions

Table 2: Distribution of Candida species on tongue site in each group

<table>
<thead>
<tr>
<th>Candida species distribution in tongue</th>
<th>Healthy subjects, ( n=5 )</th>
<th>BQC, ( n=15 )</th>
<th>OSF, ( n=15 )</th>
<th>Fisher’s exact test value</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>22.884</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>60.0</td>
<td>26.7</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>C. albicans and C. krusei</td>
<td>Count</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>0.0</td>
<td>6.7</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>C. krusei</td>
<td>Count</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>0.0</td>
<td>20.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>Count</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>0.0</td>
<td>0.0</td>
<td>60.0</td>
<td></td>
</tr>
<tr>
<td>No colonies</td>
<td>Count</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>40.0</td>
<td>46.7</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>5</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>


C. albicans due to their biofilms, greater adherence, and phenotypic switching may show an increased oral carriage in BQC with tobacco in comparison to healthy individuals without BQC habit. In the present study, an increase in the Candida carriage in samples isolated from tongue and buccal mucosa of BQC with tobacco was observed in comparison to healthy individuals without BQC. C. albicans as the major isolate is similar to previous studies which recorded similar observations on BQC.\(^{[19,22,35]}\)

C. tropicalis was isolated as a predominant isolate in OSF patients in samples collected from the tongue and buccal mucosa followed by C. albicans and C. krusei, which is in contrast to the previous studies. C. tropicalis shows the closest genetic similarity to C. albicans and exhibits similar phenotypic switching. It is also a major biofilm producer with more hexosamine content in the biofilm which makes it resistant to host immune mechanisms. C. tropicalis is also reported to be the predominant isolate in India as per geographic variation.\(^{[36-42]}\) This observation is unique to the present study as we could not find similar observations in literature in OSF patients. Similar divergence in mycotic flora has been reported in few populations which can be attributed to the influence of nutritional, social, environmental factors, and oral hygiene practices.\(^{[36,43,44]}\)

Interestingly, Reichart et al. reported that betel quid contents might not influence Candida species.\(^{[40]}\) The present observation of the switch in the Candida species, however, does not bear out this observation. It is most likely that development of OSF alters the oral environment both in the tissue and salivary component sufficient to induce the growth of C. tropicalis. Incidentally, the latter is known to proliferate in conditions of immunosuppression in the human body. Whether an element of immunosuppression known to exist in OSF was contributory to the development of the C. tropicalis is debatable. Nevertheless, the statistical significance of the predominance of C. tropicalis alludes to the change in the oral environment caused by the BQC and the subsequent development of OSF.

Cohabitation of Candida is well documented. In the present study, we isolated two cohabitating species (C. albicans and C. krusei) in one buccal mucosa sample and 3 tongue samples out of 15 OSF patients. In general, an individual has species-specific niches and harbors a specific species.\(^{[36]}\) An isolation of two species is reported in few individuals; such findings are more commonly reported in hospitalized and immunocompromised individuals.\(^{[27,46]}\)

Conclusion

An emergence of C. tropicalis as a major isolate in OSF is a unique finding in this study which may suggest a shift of Candida isolates to non-C. albicans species mainly due to host environment variation. The singular observation of this change in OSF patients is interesting and needs further corroboration. It necessitates further studies among these study groups with large sample size, species-specific confirmatory tests which may be beneficial in recognition of Candida species and treatment planning of OSF patients.

References

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Somashekhar, et al.


